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## (54) Title: CONFORMATIONALLY CONSTRAINED PEPTIDES I

## (57) Abstract

The present invention discloses compounds that inhibit HIV infection by mimicking stereochemical regions of the CD4 receptor protein.

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5

Title

CONFORMATIONALLY CONSTRAINED PEPTIDES I

10

Field of Invention

The present invention generally relates to  
15 inhibition of HIV infection by mimicking structures of  
the CD4 receptor protein.

Background of the Invention

20 The hallmark of the Acquired Immunodeficiency  
Disease, AIDS, is a progressive decline in the number of  
CD4+ cells leading to the demise of immune function and  
consequent susceptibility to opportunistic infections,  
the primary cause of death. Human immunodeficiency  
25 virus type 1 (HIV-1) is the primary causative agent of  
AIDS. This highly variable virus shows selective  
tropism for CD4+ cells which is determined by  
recognition of the HIV envelope glycoprotein gp120 by  
the CD4 cell surface receptor protein. The manner in  
30 which HIV infection leads to the slow but progressive  
decline in CD4 cells has not been established. However,  
it has been shown that an agent which antagonizes HIV in  
vitro, AZT, also provides a therapeutic benefit in vivo.  
Thus antagonism of HIV is the primary therapeutic  
35 strategy for AIDS.

The process of viral infection is initiated by  
the attachment of HIV to cells through a high affinity

interaction between gp120 and the CD4 receptor protein, located on the cell surface. This site of binding to CD4 has been localized to the first extracellular domain D1 (previously denoted as V1). A region on gp120 which 5 interacts with CD4 has also been identified but the overall structure of the protein is poorly characterized.

A second process, virus-mediated cell fusion, is also initiated by the interaction of gp120 with the 10 CD4 receptor protein. Cells infected with the HIV virus can express envelope proteins, ultimately detected on the infected-cell's surface. Thus gp120 on the surface of infected cells can bind to CD4 or uninfected cells leading to the fusion and consequent formation of 15 multinuclear giant cells (i.e., syncytium formation). This process is envisioned as a cell-cell equivalent of the binding and fusion events between HIV and an uninfected cell. However, the pathology of this virus-mediated cell fusion is not well understood.

20 Specific residues in regions of CD4 are implicated in interacting with the HIV envelope glycoproteins based upon mutational analysis, antibody inhibition and epitope studies. However, the design of compounds that will selectively block HIV infection 25 poses considerable difficulties without extensive knowledge of the molecular structures or mechanisms involved. One molecular structure has been determined, and it is thus an object of this invention to rationally design selective HIV inhibitors.

30

#### Summary of the Invention

The present invention relates to a method for inhibiting HIV infection by administering to a patient a 35 pharmaceutically effective amount of a conformationally constrained compound of the formula:

X-A-B-C-D-Y

wherein

5        A-B-C-D is a  $\beta$  turn tetrapeptide or a  $\beta$ -turn tetrapeptide mimic;

      X and Y are groups which provide a means to restrict the stereochemical structure of A-B-C-D such that A-B-C-D forms or mimics a  $\beta$ -turn; and

10      A-B-C-D binds to at least one HIV envelope protein; thereby inhibiting HIV infection.

      In related aspects, when X and Y of the present invention both comprise a naturally occurring amino acid sequence that is present in the D1 domain of the CD4 receptor protein, then the combination of X and Y taken together number less than 31 amino acids.

      This invention also relates to a conformationally restricted compound of the formula:

X-A-B-C-D-Y

20      wherein

      A is selected from the group consisting of Gly, Ala, Ser, Thr, Asp, Asn, Glu, Gln, His, Lys and Arg;

      B is any amino acid;

25      C is any amino acid;

      D is Leu, Phe or Phe-minic;

      the combination X + Y provides a means to restrict the stereochemical structure of A-B-C-D such that

30      A-B-C-D forms a beta turn;

      with the proviso that when X and Y both comprise a naturally occurring amino acid sequence present in the D1 domain of CD4, then X and Y together number less than 31 amino acids;

35      or a pharmaceutically acceptable salt thereof.

      X and Y can be chemical structures that form

covalent bonds or X and Y can be amino acids or derivatives thereof. When X and Y are amino acids, they may be joined by a disulfide bridge(s) or joined by a peptide bond(s) to restrict the stereochemical structure 5 of A-B-C-D.

In related aspects, the present invention is a recombinant protein in which the CDR-2 or CDR-3 and/or both regions of an immunoglobulin are replaced with the compounds of the present invention.

10 In further related aspects, this invention relates to a composition for inhibiting HIV infection which comprises an effective amount of the compound of the present invention and a pharmaceutically acceptable carrier.

15

#### Detailed Description of the Invention

The present invention relates to compounds which inhibit the interaction between HIV envelope 20 glycoproteins and the human cell-surface protein, CD4. In particular, they exemplify a class of molecules which inhibit binding and/or subsequent fusion by mimicking structures of the CD4 protein which interacts with HIV envelope glycoproteins (e.g., gp120, gp41, gp160). 25 These "structures" are compounds which have a restricted stereochemical conformation.

The high affinity binding site for the HIV envelope protein has been localized to the amino terminal domain of CD4, referred to as D1 (previously 30 denoted as V1). This domain was identified as the region critical for binding through expression of a truncated protein containing residues 1-106 (see, Arthos et al., *Cell*, 57:469-81 (1989)). This protein was shown to have an affinity for gp120 comparable to that of 35 soluble CD4 (i.e., native receptor).

It is believed that at least two distinct regions on D1 are involved in HIV infection of CD4<sup>+</sup>

cells. Based on mutational data and epitope mapping the region of amino acid residues 41-59 has been implicated as the high affinity binding site for gp120 (see for example, Arthos et al., Cell, 57:469-81 (1989)).

5 However, it was not clear which residues in this region are involved in binding since at many positions of a "down-effect" mutation (i.e., decrease in affinity for gp120), was countered by substitutions having little effect. Blocking of this site with specific mAbs  
10 (monoclonal antibodies) causes inhibition of gp120 binding as well as HIV infection and HIV-induced syncytium formation.

A second region, which lies outside the high affinity binding site, is thought to be required for  
15 events that occur subsequent to the initial binding step. Inhibition of this site (approximately regions 77-85) causes a limited interference of gp120 binding to CD4. Monoclonal antibodies (mAbs) that bind to this site block HIV infection of CD4<sup>+</sup> cells and the fusion  
20 between HIV infected and uninfected cells. It has also been shown that heavily benzylated peptides corresponding to this region also inhibit HIV infectivity and syncytium formation (see, for example, Lifson et al., Science, 241:712-16 (1988)).  
25 Furthermore, a single amino acid substitution resulted in loss of syncytium formation without affecting gp120 binding to CD4 (Camerini et al., Cell, 60:747-754 (1990)). The exact role of this region in the HIV-CD4 interaction is not clear. However, it does appear that  
30 this region interacts with HIV differently than the high affinity binding site.

The present invention, then, is capable of inhibiting HIV infection through: antagonism of the viral envelope glycoprotein, gp120, from binding to the  
35 CD4 receptor; or antagonism of the HIV virus - cell mediated fusion process; or antagonism of cell to cell fusion initiated by the interaction of viral envelope

protein on infected cells with the CD4 receptor on uninfected cells; or a combination of any of the above.

A more detailed structure of the amino-terminal domains of CD4 has since been determined. A high 5 resolution crystal structure of D1D2 (previously denoted as V1V2) indicates that it is overall of the  $\beta$ -type. That is, the backbone of the polypeptide chain is in a  $\beta$  conformation, where the polypeptide  $\beta$  strands are arranged side by side in an anti-parallel direction and 10 form interstrand hydrogen bonds. D1D2 comprises two associated domains. D1 (previously denoted as V1), which approximately comprises residues 1 to 98, is arranged into nine  $\beta$  strands. The second domain D2 (residues 99 to 173), comprises seven  $\beta$  strands.

15 The beta strands of D1 are connected by loops at the end of the beta strands (i.e., beta turn loops). That is, a beta or beta turn loop consists of two antiparallel  $\beta$ -strands and a beta turn. Some of these "loops" comprise hairpin structures having a beta turn 20 of approximately 4 amino acids denoted as i, i+1, i+2 and i+3. Based upon structural similarity, three of the loops are analogous to the complementarity determining regions (CDR) of the immunoglobulin variable-light domains (Ig-V<sub>L</sub>). One beta turn loop (defined by  $\beta$  strands C<sup>1</sup> and C<sup>11</sup> or approximately residues 37-46), 25 corresponds to CDR2 of an Ig variable region. Another beta turn loop (defined by  $\beta$  strands F and G or approximately residues 79-96), corresponds to CDR3 of an Ig variable region.

30 In D1, a hairpin loop comprising a  $\beta$ -turn at residues Gln<sup>40</sup> to Phe<sup>43</sup> protrudes from the D1D2 molecule. Furthermore, the hydrophobic side chain of Phe<sup>43</sup> appears to project away from D1D2. A similar structure is found for residues Val<sup>86</sup> to Gln<sup>89</sup>, that is, 35 a hairpin loop that protrudes from D1D2.

One embodiment of the present invention are peptides or peptides mimics that are conformationally

constrained to stabilize a two-stranded antiparallel beta sheet with a beta turn. The structure of the beta turn is herein referred to as A-B-C-D which corresponds to the  $i$ ,  $i+1$ ,  $i+2$  and  $i+3$  positions as previously 5 denoted. Therefore, A-B-C-D is a  $\beta$  turn or  $\beta$  turn mimic in the present invention. The structure of the beta turn of a constrained peptide may be verified via structural analysis, e.g. NMR or X-ray crystallography. Such peptides or peptide mimics can have a range in 10 length from 4 to 100, preferably 4 to 75, and more preferably 6 to 30.

When A, B, C and D are all amino acids, the  $\beta$  turn is herein referred to as a  $\beta$  turn tetrapeptide. When the peptide backbone of at least one amino acid is 15 replaced by a non-peptide frame capable of carrying the amino acid side-chain group(s) in a conformation that mimics that of a  $\beta$  turn, the structure is referred to as a  $\beta$  turn tetrapeptide mimic. Such  $\beta$  turn mimics have at least one non-peptidyl bond, e.g., an acyl hydrazide 20 linkage. Another example of  $\beta$  turn mimics is reported by Olson et al. (J Am Chem Soc, 112:323-333 (1990)) and is incorporated by reference herein.

As used herein, the term "conformationally constrained" or "conformationally restricted" refers to 25 limitations imposed on the number of possible stereochemical structures that a peptide or peptide mimic may assume. In the present invention, a beta turn or beta turn mimics are formed. Such restrictions are imposed on the conformation (or stereochemical 30 structure) by the presence of chemical structures or groups, herein referred to as "X" and "Y", (e.g., amino acid or other chemical moieties) surrounding the beta turn. Where specific chemical structures or sequences 35 are presented, it is intended to encompass various modifications (e.g., acetylation, alkylation, etc.) which do not destroy the inhibitory functions of the present invention.

For example, conformational stabilization can be achieved by covalently linking residues on either side of the desired beta turn at specific positions with groups that stabilize both the antiparallel beta structure and the desired position in the sequence of the turn.

It is preferable, but not absolute, that the compounds of the present invention are peptides. Such restrictions can be accomplished by covalently linking residues at specific positions to conformationally constrain or restrict the compounds of the present invention. For example, this includes, but is not limited to, formation of disulfide bonds, homodetic peptide cyclization, and attachment to larger protein molecules.

One preferred embodiment comprises residues 40 (residue "i" of the beta turn or "A") through 43 (residue "i+3" or "D") of the D1 domain of CD4. Preferably, residue 43 (i.e., "D" or the i+3 position) 20 is leucine or phenylalanine or a phenylalanine mimic, e.g., tyrosine, tryptophan, 3-thienylalanine, naphthylalanine or phenylalanine wherein the phenyl group is substituted by halogen, C<sub>1-4</sub> alkyl or C<sub>1-4</sub> alkoxy.

"A" is preferably Gly, Ala, Ser, Thr, Asp, Asn, Glu, Gln, His, Lys or Arg. Another preferred embodiment are conformationally constrained peptides which comprise residues 86 (i.e., i or "A") through 89 (i+3 or "D"). In this embodiment "B" is preferably Gly, Ala, Ser, Thr, 30 Asp, Asn, Glu, Gln, His, Lys or Arg).

For purposes of illustration, the beta hairpin may be shown schematically as follows:

N-term.

35 -Lys<sup>35</sup>-Ile<sup>36</sup>-Leu<sup>37</sup>-Gly<sup>38</sup>-Asn<sup>39</sup>-Gln<sup>40</sup>-Gly<sup>41</sup>  
-Pro<sup>48</sup>-Gly<sup>47</sup>-Lys<sup>46</sup>-Thr<sup>45</sup>-Leu<sup>44</sup>-Phe<sup>43</sup>-Ser<sup>42</sup>

C-term.

N-term.

-Ser<sup>79</sup>-Asp<sup>80</sup>-Thr<sup>81</sup>-Tyr<sup>82</sup>-Ile<sup>83</sup>-Cys<sup>84</sup>-Glu<sup>85</sup>-Val<sup>86</sup>-Glu<sup>87</sup>

5

-Leu<sup>76</sup>-Leu<sup>95</sup>-Gln<sup>94</sup>-Val<sup>93</sup>-Glu<sup>92</sup>-Glu<sup>91</sup>-Lys<sup>90</sup>-Gln<sup>89</sup>-Asp<sup>88</sup>

C-term.

Wherein the beta turn comprises residues 40 through 43, or  
10 residues 86-89.

The methods for stabilizing the antiparallel beta hairpin conformation may include, but are not limited to, formation of disulfide bonds, homodetic peptide cyclization, a combination of disulfide bonds and peptide cyclization, or  
15 attachment to larger protein molecules.

For example, the incorporation of cysteine residue pairs are positioned such that, when they are linked (by oxidation of their side chains) to form a disulfide bond, an antiparallel structure is stabilized. Preferred embodiments  
20 include Cys pairs at positions 39,44; at 38,45; at 37,46; at 36,47; at 35,48; at 85,90; at 84,91; at 83,92; at 82,93; at 81,94; at 80,95; and at 79,96. Exemplars of conformational CD4 mimics are depicted below:

25 N-term.

-Cys-Gln<sup>40</sup>-Gly<sup>41</sup>

\*

-Cys-Phe<sup>43</sup>-Ser<sup>42</sup>

C-term.

30 Wherein \* indicates a disulfide bond between the sulphydryl groups of cysteine.

N-term.

-Cys-Asn<sup>39</sup>-Gln<sup>40</sup>-Gly<sup>41</sup>

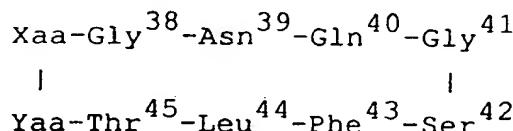
35

\* -Cys-Leu<sup>44</sup>-Phe<sup>43</sup>-Ser<sup>42</sup>

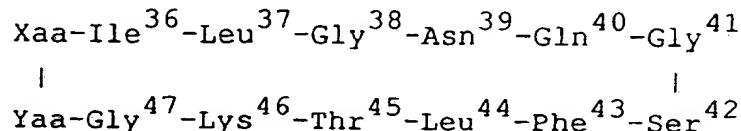
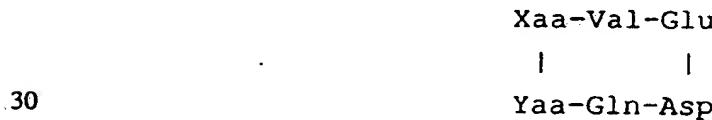
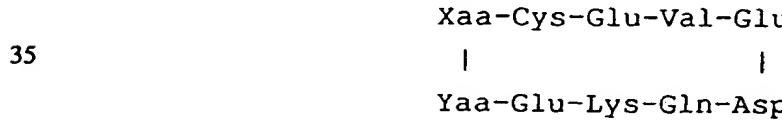
C-term.

5	<u>N-term.</u>	-Cys-Gly <sup>38</sup> -Asn <sup>39</sup> -Gln <sup>40</sup> -Gly <sup>41</sup>
		*
		-Cys-Thr <sup>45</sup> -Leu <sup>44</sup> -Phe <sup>43</sup> -Ser <sup>42</sup>
	<u>C-term.</u>	
10	<u>N-term.</u>	-Cys-Leu <sup>37</sup> -Gly <sup>38</sup> -Asn <sup>39</sup> -Gln <sup>40</sup> -Gly <sup>41</sup>
		*
		-Cys-Lys <sup>46</sup> -Thr <sup>45</sup> -Leu <sup>44</sup> -Phe <sup>43</sup> -Ser <sup>42</sup>
	<u>C-term.</u>	
15	<u>N-term.</u>	-Cys-Val-Glu
		*
		-Cys-Gln-Asp
	<u>C-term.</u>	
20	<u>N-term.</u>	-Cys-Glu-Val-Glu
		*
		-Cys-Lys-Gln-Asp
	<u>C-term.</u>	
25	<u>N-term.</u>	-Cys-Cys-Glu-Val-Glu
		*
		-Cys-Glu-Lys-Gln-Asp
30	<u>C-term.</u>	
	<u>N-term.</u>	-Cys-Ile-Cys-Glu-Val-Gln
		*
		-Cys-Glu-Glu-Lys-Gln-Asp
35	<u>C-term.</u>	

Another example of conformational restriction is by homodetic peptide cyclization using beta turn favoring amino acid pairs to form a cyclic peptide. Preferably, the cyclic peptide comprises two beta turns connecting extended antiparallel beta segments. Such a backbone cyclization should preferably form rings comprising  $4n+2$  residues, e.g., 6, 10, 14, etc. residues. Exemplars are depicted below:

N-term.C-term.15    N-term.C-term.

20

N-term.25    C-term.N-term.C-term.N-term.C-term.

N-term.

Xaa-Tyr-Ile-Cys-Glu-Val-Glu

|

|

5 Yaa-Val-Glu-Glu-Lys-Gln-Asp

C-term.

The sequence Yaa-Xaa stabilizes this turn and may include such pairs as Pro-D-Pro, D-Pro-Pro (where Pro 10 may be replaced by a proline analog such as beta, beta dimethyl gamma-thiaproline), or pairs with only one proline, e.g., D-Zaa-L-Pro or Zaa-D-Pro, where Zaa is any other amino acid (or modified amino acid) capable of forming a peptide bond to Pro.

15 As used herein, the term "peptide bond" refers to an amide linkage between a carboxyl group of one amino acid and the a-amino group of another amino acid.

In another embodiment, the sequence Gly<sup>41</sup>-Ser<sup>42</sup> or Glu<sup>87</sup>-Asp<sup>88</sup> may similarly be replaced at the same 20 time to further stabilize the desired structure. For example:

N-term.Xaa-Ile<sup>36</sup>-Leu<sup>37</sup>-Gly<sup>38</sup>-Asn<sup>39</sup>-Gln<sup>40</sup>-Yaa'

25

|

|

Yaa-Gly<sup>47</sup>-Lys<sup>46</sup>-Thr<sup>45</sup>-Leu<sup>44</sup>-Phe<sup>43</sup>-Xaa'C-term.N-term.

30

Xaa-Cys<sup>84</sup>-Glu<sup>85</sup>-Val<sup>86</sup>-Yaa'

|

|

Yaa-Glu<sup>91</sup>-Lys<sup>90</sup>-Gln<sup>89</sup>-Xaa'C-term.

35 where Xaa' and Yaa' are the same or different turn stabilizing pairs as Xaa and Yaa.

Peptides may also be conformationally

restricted by a combination of homodetic peptide cyclization and disulfide bonds. For example, the Gly<sup>41</sup>-Ser<sup>42</sup> or Glu<sup>87</sup>-Asp<sup>88</sup> sequence is replaced by a turn stabilizing sequence (see above) and a Cys-Cys 5 disulfide bond is incorporated to comprise a homodetic cyclic peptide which has a stabilizing disulfide link. For example:

N-term.

10 Xaa-Ile<sup>36</sup>-Leu<sup>37</sup>-Cys-Asn<sup>39</sup>-Gln<sup>40</sup>-Yaa'  
| \* |  
Yaa-Gly<sup>47</sup>-Lys<sup>46</sup>-Cys-Leu<sup>44</sup>-Phe<sup>43</sup>-Xaa'

C-term.

15 N-term.

Xaa-Tyr<sup>82</sup>-Ile<sup>83</sup>-Cys<sup>84</sup>-Glu<sup>85</sup>-Val<sup>86</sup>-Glu<sup>87</sup>  
| \* |  
Yaa-Val<sup>93</sup>-Glu<sup>92</sup>-Cys<sup>91</sup>-Lys<sup>90</sup>-Gln<sup>89</sup>-Asp<sup>88</sup>

C-term.

20

Conformationally restrained peptides can also be stabilized by attachment to larger protein molecules, such as an immunoglobulin. For example, all or a portion of the CDR-2 region from the CD4 receptor 25 protein (approximately residues 37 to 46) can be substituted for the structurally analogous loop of an immunoglobulin. Preferably the immunoglobulin is a human IgG subclass. More preferably it comprises a variable light chain, e.g., the variable light chain 30 (V<sub>k</sub>) of the Bence-Jones REI protein (residues 48-54) [See Sattentau et al., J Exp Med, 170:1319-34 (1989) or Epp et al., Biochem, 14:4943 (1975)], resulting in the sequence below wherein residues from CD4 are underlined:

35 (N-term) Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu  
Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gln Ala  
Ser Gln Asp Ile Ile Lys Tyr Leu Asn Trp Tyr Gln Gln Thr

15 Pro Gly Lys Ala Pro Lys Leu Leu Leu Gly Asn Gln Gly Ser  
Phe Leu Thr Lys Gln Ala Gly Val Pro Ser Arg Phe Ser Gly  
Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu  
Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Gln  
5 Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile  
Thr (C-term)

10 In another embodiment, the CDR-3 region of the  
CD4 receptor protein (residues 81 to 93) can be  
substituted for the structurally analogous loop in the  
Bence-Jones REI protein (residues 85-102), resulting in  
the sequence below wherein residues from CD4 are  
underlined:

15 (N-Term) Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu  
Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gln Ala  
Ser Gln Asp Ile Ile Lys Tyr Leu Asn Trp Tyr Gln Gln Thr  
Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Glu Ala Ser Asn  
Leu Gln Ala Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser  
20 Gly Thr Asp Tyr Thr Phe Thr Ile Thr Ile Ser Ser Leu Gln  
Pro Glu Asp Ile Ala Thr Tyr Ile Cys Glu Val Glu Asp Gln  
Lys Glu Glu Val Lys Leu Gln Ile Thr (C-term)

25 In still another embodiment, the compounds of  
the present invention can be constructed by substituting  
the CDR-2 and CDR-3 residues of CD4 for the structurally  
analogous loops in the Bence-Jones REI protein (residues  
48-54 and 85-102, respectively), resulting in the  
sequence below wherein the residues from CD4 are  
30 underlined:

(N-term) Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu  
Ser Ala Ser Val Gly Asp Arg Val Thu Ile Thr Cys Gln Ala  
Ser Gln Asp Ile Ile Lys Tyr Leu Asn Trp Tyr Gln Gln Thr  
35 Pro Gly Lys Ala Pro Lys Leu Leu Leu Gly Asn Gln Gly Ser  
Phe Leu Thr Lys Gln Ala Gly Val Pro Ser Arg Phe Ser Gly  
Ser Gly Ser Gly Thr asp Tyr Thr Phe Thr Ile Ser Ser Leu

Gln Pro Glu Asp Ile Ala Thr Tyr Ile Cys Glu Val Glu Asp  
Gln Lys Glu Glu Val Lys Leu Gln Ile Thr (C-term)

Peptides of the present invention may be  
5 synthesized by any suitable method. Methods of well-known peptide synthesis are generally set forth by Ali et al., J Med Chem, 29:984 (1986) and J Med Chem, 30:2291 (1987) and are incorporated by reference herein. Preferably, the peptides are prepared by the solid phase  
10 technique of Merrifield (J Am Chem Soc, 85:2149 (1964)). However, a combination of solid phase and solution synthesis may be used, as in a convergent synthesis in which di-, tri-, tetra-, or penta-peptide fragments may be prepared by solid phase synthesis and either coupled  
15 or further modified by solution synthesis.

During synthesis, the side chain functional groups (e.g., -NH<sub>2</sub>, -COOH, -OH, -SH) are protected during the coupling reactions. Normally, the  $\alpha$ -amino group is temporarily protected as t-Butoxycarbonyl (BOC) but other acid or base labile protecting groups can be used, e.g., fluorenylmethoxycarbonyl (FMC). The amino side chain group of lysine is protected as benzyloxycarbonyl or p-chlorobenzyloxycarbonyl (Z or Cl-Z). Para-methylbenzyl (p-MBz) or acetomidomethyl protection is used for cysteines. Hydroxy groups are protected as benzyl ethers and carboxyl groups are protected as benzyl (Bz) or cyclohexyl esters.

The peptides of the present invention can be synthesized either from the C-terminus or the N-terminus. Preferably it is from the C-terminus. Prior to coupling the alpha-carboxyl group (of a suitable protected amino acid) is activated. One skilled in the art can activate the protected group in a number of ways. For example, one may use N,N'  
30 dicyclohexylcarbodiimide (DCC), p-nitrophenyl esters (pNp), hydroxybenzotriazole ester (HOBT), N-hydroxy  
35 succinimidyl ester (Osu) mixed anhydride or symmetrical

anhydride.

Solution synthesis of peptides is accomplished using conventional methods to form amide bonds.

Typically, a protected Boc-amino acid which has a free carboxyl group is coupled to a protected amino acid which has a free amino group using a suitable carbodiimide coupling agent, such as N, N' dicyclohexyl carbodiimide (DCC), optionally in the presence of a catalyst such as 1-hydroxybenzotriazole (HOBT) and 10 dimethylamino pyridine (DMAP).

In solution phase synthesis, the coupling reactions are preferably carried out at low temperature (e.g., -20°C) in such solvents as dichloromethane (DCM), dimethyl formamide (DMF), N-methyl pyrrolidone (NMP), 15 tetrahydrofuran (THF) acetonitrile (ACN) or dioxane.

If solid phase methods are used, the peptide is built up sequentially starting from the carboxy terminus and working towards the amino terminus of the peptide. Solid phase synthesis begins by covalently attaching the 20 C terminus of a protected amino acid to a suitable resin, such as methyl benzhydrylamine (mBHA).

In the solid phase synthesis, the first amino acid residue is normally attached to an insoluble polymer. For example, two commonly used polymers are 25 polystyrene (1% cross-linked with divinyl benzene) and 1% cross-linked polyamides. These polymers are functionalized to contain a reactive group, e.g., -OH, -NH<sub>2</sub> and -CH<sub>2</sub>Cl to link the first amino acid of the targeted peptide (i.e., carboxy terminus). The choice 30 of the linkage between the first amino acid and the polymer is dictated by the carboxy terminus of the peptide. For example, peptides having a carboxyl group at the C-terminus would be linked by an ester linkage and for peptides with a carboxamide ending would have an 35 amide linkage.

Once the first protected amino acid has been coupled to the desired resin, the protected amino

group is hydrolyzed by mild acid treatment, and the free carboxyl of the next (protected) amino acid is coupled to this amino group. This process is carried out sequentially, without isolation of the intermediate, 5 until the peptide of interest has been formed. The completed peptide may then be deblocked and/or cleaved from the resin in any order.

Preferred solvents for the coupling reactions include, but are not limited to, dichloromethane (DCM), 10 dimethyl formamide (DMF) and N-methyl pyrrolidone (NMP). After the desired sequence is synthesized, the peptide is deprotected and cleaved from the resin using standard techniques (e.g., using hydrofluoric acid (HF)).

The preferred method for cleaving a peptide 15 from the support resin is to treat the resin supported peptide with anhydrous HF in the presence of a suitable cation scavenger, such as anisole or dimethoxy benzene.

To obtain the conformationally restricted peptides of the present invention, the synthetic peptide 20 may be cyclized using methods well known in the art. For example, there are numerous protocols for forming intramolecular bonds between cysteine residues. In performing these reactions, water, methanol, acetic acid, DMF or a suitable mixture of these solvents can be 25 used.

Formation of the disulfide bond may be accomplished by several known methods. If the sulfur-containing amino acids of the linear peptide are protected differently, in such a manner as to allow 30 formation of a mono mercaptan, cyclization may be effected by base catalyzed nucleophilic displacement of the protecting group of the sulfur-containing amino acid. Groups which are especially useful as displaceable protecting groups are thioalkyl or thioaryl 35 groups. Exemplary of this method is the protection of one sulfur-containing amino acid by the thioethyl group, and protection of the second by a substituted benzyl

group. Deprotection of such a peptide by HF removes the benzyl group from one amino acid, while leaving the second protected as an ethyl disulfide. Stirring this mercapto- $\beta$ -disulfide in dilute solution at a pH of about 7 to 8 effects displacement of the thioethyl group and cyclization of the linear peptide. When cysteine is protected with an ACM (acetamido methyl) group the preferred method is to oxidize with iodine.

If the corresponding linear peptide is completely deprotected and produced as a dimercaptan, any oxidizing agent known in the art to be capable of converting a dimercaptan to a disulfide may be used. Exemplary of such agents are an alkali metal ferricyanide, (e.g., potassium or sodium ferricyanide), oxygen gas, diiodomethane or iodine. The reaction is conducted in a suitable inert solvent, such as aqueous methanol or water, at temperatures from about 0 to 40°C, under high dilution. The pH is usually maintained at about 7 to 8. Cyclization may be performed upon the peptide while it is still attached to the support resin or while other functional groups are still protected, but it is preferably performed on the deprotected free peptide.

The conformationally restricted peptides can also be prepared by forming a peptide bond between non-adjacent amino acid residues. One procedure for forming peptide bonds is provided by Schiller et al., Int J Peptide Protein Res, 25:171 (1985).

Acid addition salts of the peptides are prepared in a standard manner in a suitable solvent from the parent compound and an excess of an acid, such as hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, maleic, succinic, or methanesulfonic. The acetate salt form is especially useful. Certain of the compounds form inner salts or zwitterions which may be acceptable. Cationic salts are prepared by treating the parent compound with an excess of an alkaline reagent, such as

a hydroxide, carbonate or alkoxide containing the appropriate cation. Cations such as  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{NH}_4$  are examples of cations present in pharmaceutically acceptable salts.

5 The peptides can then be purified by a number of techniques. Preferred embodiments include reverse phase HPLC, counter current distribution (CCD) and crystallization. More preferably, HPLC is used. The purified products can then be analyzed for purity using  
10 HPLC, amino acid analysis and fast atom bombardment mass spectrometry (FAB-MS).

The compounds of the present invention can be assayed for their ability to inhibit HIV infection via binding assays and functional (fusion or infectivity) assays. One binding assay entails a competition ELISA, measuring the binding inhibition of st4 to immobilized recombinant gp120 in the presence and absence of the compounds of the invention. A similar competition RIA entails measuring the binding inhibition of labelled st4 to (immobilized) recombinant gp120. Another method entails the inhibition of HIV gp120 binding to CD4+ cells. Bound gp120 is detected by gamma counting when using  $^{125}\text{I}$ -labelled gp120 or by glow cytometry when using FITC (fluorescein isothiocyanate)-labelled gp120.  
25 Alternatively, anti-gp120 antibodies may also be used to detect binding. These assays are performed essentially as described in Arthos et al. (Cell, 57:469-81 (1989)) and Sattentau et al. (J Exp Med, 170:1319-34 (1989)), and are incorporated by reference herein. It is noted  
30 that the compounds of the present invention bind to HIV envelope proteins with the same or greater affinity than the native CD4 or soluble CD4 proteins.

One functional assay (i.e., syncytium assay) comprises the inhibition of cell fusion between  
35 chronically infected cells and uninfected  $\text{CD4}^+$  cells. In essence, HIV infected H9 cells (R. Gallo, National Institute of Health, Bethesda, MD, USA) are cocultivated

overnight with uninfected cells at a ratio of 1:2, in the presence of an inhibitory substance&compound. Such assays are performed essentially as described by Sleckman et al., (Nature, 328:351-3 (1987)) and are

5 hereby incorporated by reference.

A non-viral syncytium assay can also be used. This assay measures the inhibition of fusion between cells expressing HIV env protein and CD4<sup>+</sup> cells as disclosed in U.S application serial number, 07/8587,011, 10 filed September 24, 1990 (Clark et al., "Human Lymphoid Cells Expressing HIV Envelope Protein gp160") and incorporated by reference herein.

Another functional assay is the virus infectivity assay. This assay comprises infection of T-lymphocytes or macrocyte&macrophages with HIV. At six 15 or more days post-infection measurement of particle-associated reverse transcriptase activity and&or p24 antigen levels can be determined (See, for example, Clapham et al., Nature, 337: 368-370 (1990) or McDougal et al., J Immun Meth, 76: 171-183 (1985)).

Pharmaceutical compositions of the peptides prepared as hereinbefore described and other peptide or polypeptide derivatives may be formulated as solutions of lyophilized powders for parenteral administration. 25 Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. The liquid formulation is generally a buffered, isotonic, aqueous solution. Examples of suitable diluents are normal isotonic saline solution, 30 standard 5% dextrose in water or buffered sodium or ammonium acetate solution. Such formulation is especially suitable for parenteral administration, but may also be used for oral administration or contained in a metered dose inhaler or nebulizer. It may be 35 desirable to add excipients such as polyvinylpyrrolidone, gelatin, hydroxy cellulose, acacia, polyethylene glycol, mannitol, sodium chloride

or sodium citrate.

Alternatively, these peptides may be encapsulated, tableted or prepared in a emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. Liquid carriers include syrup, peanut oil, olive oil, saline and water. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies but, preferably, will be between about 20mg to about 1g per dosage unit. The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing, granulating, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly or filled into a soft gelatin capsule.

For rectal administration, the peptides of this invention may also be combined with excipients such as cocoa butter, glycerin, gelatin or polyethylene glycols and molded into a suppository.

The dosage ranges for administration of the compounds of the present invention are those to produce the desired effect whereby symptoms of HIV or HIV infection are ameliorated. As used herein, a pharmaceutically effective amount refers to the amount administered so as to maintain an amount which suppresses or inhibits secondary infection by syncytia formation or by circulating virus throughout the period

during which HIV infection is evidenced such as by presence of anti-HIV antibodies, presence of culturable virus and presence of p24 antigen in patient sera. The presence of anti-HIV antibodies can be determined 5 through use of standard ELISA or western assays for example, anti-gp120, anti-gp41, anti-tat, anti-p55, anti-p17 antibodies, etc. The dosage will generally vary with age, extent of the infection, and counterindications, if any, for example, immune 10 tolerance. The dosage can vary from 0.001 mg/kg/day to 50 mg/kg/day, but preferably 0.01 to 1.0 mg/kg/day.

The Examples which follow are illustrative, and not to be construed as limiting of the present invention.

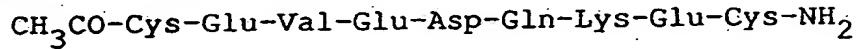
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#### EXAMPLES

In the examples which follow all temperatures are in degrees centigrade. Amino acid analysis was 20 performed upon a Dionex autoion 100. Mass spectra were performed upon a VG Zab mass spectrometer using fast atom bombardment. HPLC was performed upon a Beckman 344 gradient chromatography system with a CRIB recording integrator in either an isocratic or continuous gradient 25 mode. Where indicated, the purity of the peptide is based upon integration of the HPLC chromatogram.

In accordance with conventional representation, the amino terminus is on the left and the carboxy terminus is on the right. Unless specified otherwise, 30 all amino acids are assumed to be of the L- configuration.

##### Example 1. Preparation of:



35

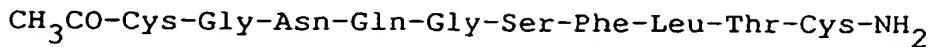
A manual shaker vessel was charged with 1.45g of methylBHA resin (substitution 0.7 mM<sup>2</sup>g). The resin

was washed with 40% trifluoroacetic acid (TFA) in dichloromethane (DCM). The resulting trifluoroacetate salts were neutralized with 10% diisopropylethyl amine (DIEA) in DCM. After neutralization, BOC-Cys (P-MBz)-5 COOH was coupled to the resin using dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBT) in 50% DMF $\beta$ DCM (10 ml). Three equivalents of amino acid, DCC and HOBT were used. The coupling, monitored using Kaiser's test, is normally complete in 10 two hours. After coupling, the resin was extensively washed with DCM and NMP, the cycle of deprotection, neutralizaiton and coupling step was repeated with the remaining amino acids in the target peptide sequence. Each coupling was monitored for completion using the 15 Kaiser test. After the coupling of the last amino acid, the resin was subjected to a deprotection and neutralization cycle and the resulting free amino group was acetylated using 10% acetic anhydride in pyridine. The peptide was cleaved from the resin using 10 ml HF 20 and 1 ml anisole at -15 $^{\circ}$ C for two hours. After removal the resin-peptide mixture was extensively washed with ether. The peptide was extracted in glacial acetic acid. The extract was diluted with water so that, the final concentration of the peptide was about 500 mg $\beta$ l. 25 The pH was adjusted to 7.5 and the solution was stirred at room temperature. Formation of the disulfide bond was monitored using Ellman's reagent. Upon completion of oxidation, the solvent was removed on a rotovap. The crude peptide was purified on a preparative C-18 (2 $\times$ 25 30 cm) HPLC Vydac column using 0.1% TFA $\beta$ H<sub>2</sub>O and a 0.1% TFA $\beta$ CH<sub>3</sub>CN gradient. It was analyzed for amino acid composition and molecular weight.

HPLC purity: >95%

Amino acid analysis:

35 Asp 1.01 (1), Lys 1.01 (1), Glu $\beta$ Gln 4.03 (4)  
and Val 1.00 (1)  
M+H 1121.5

Example 2. Preparation of:

[ ]

5 An ABI model 430 peptide synthesizer was charged with 700 mg of methyl BHA resin (substitution 0.7 mM $\text{Mg}$ ). A standard protocol (provided by Applied Bio System Inc. Foster City, CA.) was used to couple the amino acids in the target peptide sequence. After 10 coupling the last amino acid, the resin was subjected to a deprotection and neutralization cycle and resulting free amino group was acetylated using 10% acetic anhydride in pyridine. The peptide was cleaved from the resin using 10 ml HF and 1 ml anisole at -15°C for two 15 hours. HF was removed; the peptide-resin mixture was extensively washed with ether and the peptide was extracted in glacial acetic acid. The acetic acid was diluted with water so that the final concentration of the peptide was about 500 mg $\text{Ml}$ . The pH was adjusted to 20 7.5 and solution was stirred at room temperature. Formation of the disulfide bond was monitored using Ellman's reagent. Once the disulfide formation was complete the solvent was removed on a rotovap. The crude peptide was purified on a preparative C-18 (2x25 25 cm) HPLC Vydac column using 0.1% TFA $\text{Mg}_2\text{O}$  and a 0.1% TFA $\text{Mg}_2\text{CH}_3\text{CN}$  gradient. It was analyzed for amino acid composition and molecular weight.

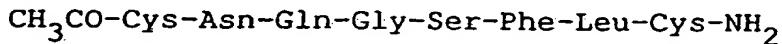
HPLC purity: >95%

Amino acid analysis:

30 Asn 1.00 (1), Gln 0.97 (1), Gly 2.02 (2), Leu 1.03 (1), Phe 1.01 (1), Ser 0.79 (1), and Thr 0.88 (1).

M+H 1071.3

35. Example 3. Preparation of:



[ ]

An ABI model 430 peptide synthesizer was charged with 700 mg of methyl BHA resin (substitution 0.5 mM $\beta$ g). A standard protocol provided by (Applied Bio Systems Inc. Foster City, CA.) was used to couple the 5 amino acids in the target peptide sequence. In this synthesis, -SH group of cysteine was protected as ACM. After coupling the last amino acid, the resin was subjected to a deprotection and neutralization cycle and the resulting free amino group was acetylated using 10% 10 acetic anhydride in pyridine. The peptide was cleaved from the resin using 10 ml HF and 1 ml anisole at -15°C for two hours. After removal of HF, the resin was extensively washed with ether and the peptide extracted in 80% acetic acid. The final concentration of the 15 peptide was about 500 mg $\beta$ l. Iodine solution (10% in 80% Acetic acid) was titrated into the peptide solution so that the solution stayed a very faint pale yellow. Formation of the disulfide was monitored using Ellman's reagent. Once the disulfide formation was complete the 20 solvent was removed on a rotovap. The crude peptide was purified on a preparative C-18 (2x25 cm) HPLC Vydac column using 0.1%TFA $\beta$ H<sub>2</sub>O and a 0.1%TFA $\beta$ CH<sub>3</sub>CN gradient. The peptide was analyzed for amino acid composition and 25 molecular weight.

HPLC purity: >95%

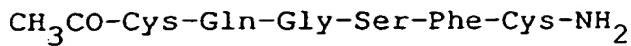
Amino acid analysis:

Asn 1.00 (1), Gly 1.02 (1), Gln 0.97 (1), Leu 1.07 (1), Phe 1.12 (1) and Ser 0.77 (1)  
M+H 910.3

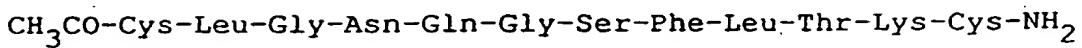
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Example 4. Additional Peptides

In addition to the peptides disclosed in Examples 1-3, the following peptides were synthesized 35 using the methods described above:  
C'-C" loop (CDR-2 like) mimics.

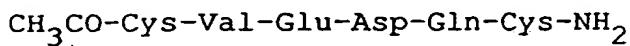


and

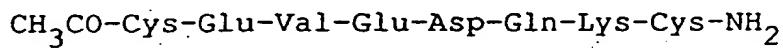


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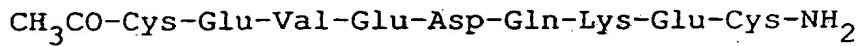
F-G loop (CDR-3 like) mimics.



10 and



and

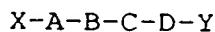


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The above examples and description fully disclose the present invention, including preferred embodiments thereof. This invention, however, is not limited to the precise embodiments described herein, but encompasses 20 all modifications within the scope of the claims which follow.

What is claimed is:

1. A method to inhibit HIV infection which comprises administering to a patient a pharmaceutically effective amount of a conformationally constrained compound of the formula:



wherein

10 A-B-C-D is a  $\beta$  turn tetrapeptide or a  $\beta$ -turn tetrapeptide mimic;

X and Y are groups which provide a means to restrict the stereochemical structure of A-B-C-D such that A-B-C-D forms or mimics a  $\beta$ -turn; and

15 A-B-C-D binds to at least one HIV envelope protein; thereby inhibiting HIV infection, with the proviso that when X and Y both comprise a naturally occurring amino acid sequence present in the D1 domain of CD4, then X and Y together number less than 31 amino acids.

20

2. The method of claim 1 wherein:

A is selected from the group consisting of Gly, Ala, Ser, Thr, Asp, Asn, Glu, Gln, His, Lys and Arg;

B is any amino acid;

25 C is any amino acid; and

D is Leu, Phe or a Phe-mimic.

30 3. The method of claim 2 wherein X and Y number less than 13 amino acids.

35

4. The method of claim 2 wherein X and Y number less than 5 amino acids.

35 5. The method of claim 2 wherein X and Y number less than 3 amino acids.

6. The method of claim 2 wherein D is Phe.

7. The method of claim 2 wherein A is Gln.

8. The method of claim 2 wherein B is Gly.

5

9. The method of claim 2 wherein C is Ser.

10. The method of claim 2 wherein A-B-C-D is  
Gln-Gly-Ser-Phe.

10

11. A conformationally restricted compound of  
the formula:

X-A-B-C-D-Y

wherein

15 A is selected from the group consisting of Gly,  
Ala, Ser, Thr, Asp, Asn, Glu, Gln, His, Lys and Arg;

B is any amino acid;

C is any amino acid;

D is Leu, Phe or Phe-minic;

20 the combination X + Y provides a means to  
restrict the stereochemical structure of A-B-C-D such  
that

A-B-C-D forms a beta turn;

with the proviso that when X and Y both

25 comprise a naturally occurring amino acid sequence  
present in the D1 domain of CD4, then X and Y together  
number less than 31 amino acids;

or a pharmaceutically acceptable salt thereof.

30 12. The compound of claim 11 wherein D is Phe.

13. The compound of claim 11 wherein A is Gly,  
Ala, Ser, Asn or Gln.

35 14. The compound of claim 11 wherein A is Gln.

15. The compound of claim 11 wherein B is Gly,

Ala, Val, Ser or Thr.

16. The compound of claim 11 wherein B is Gly.

5 17. The compound of claim 11 wherein C is Gly,  
Ala, Val, Ser or Thr.

18. The compound of claim 11 wherein C is Ser.

10 19. The compound of claim 11 wherein A-B-C-D is  
Gln-Gly-Ser-Phe.

20. The compound of claim 11 wherein X is one  
or more amino acids and Y is one or more amino acids.

15 21. The compound of claim 20 wherein X and Y  
are joined by a disulfide bridge.

22. The compound of claim 21 wherein:  
20

X is Cys-(X<sub>1</sub>)<sub>n</sub>;  
Y is Cys-(Y<sub>1</sub>)<sub>n</sub>;  
(X<sub>1</sub>) is any amino acid except Pro;  
(Y<sub>1</sub>) is any amino acid except Pro;  
25 and n = 0 to 5.

23. The compound of claim 21 wherein:  
X is selected from the group consisting of  
Xaa<sub>n</sub>-Cys-, Xaa<sub>n</sub>-Cys-Asn-, Xaa<sub>n</sub>-Cys-Gly-Asn- and Xaa<sub>n</sub>-  
30 Cys-Leu-Gly-Asn-;  
Y is selected from the group consisting of  
Yaa<sub>n</sub>-Cys-, Yaa<sub>n</sub>-Cys-Leu-, Yaa<sub>n</sub>-Cys-Thr-Leu and

$Yaa_n$ -Cys-Lys-Thr-Leu-;

Xaa is any amino acid except Pro;

Yaa is any amino acid except Pro;

and n = 0 to 11.

5

24. The compound of claim 20 wherein X and Y are joined by a peptide bond.

25. The compound of claim 24 wherein:

10 X is selected from the group consisting of

$Xaa_n$ -,  $Xaa_n$ -Gly-Asn- and  $Xaa_n$ -Ile-Leu-Gly-Asn-;

Y is selected from the group consisting of

$Yaa_n$ -,  $Yaa_n$ -Thr-Leu- and  $Yaa_n$ -Gly-Lys-Thr-Leu;

15 Xaa is any amino acid capable of forming a peptide bond with Yaa;

Yaa is any amino acid capable of forming a peptide bond with Xaa;

and n = 1 to 11.

20 26. The compound of claim 25 wherein the pair Xaa-Yaa comprises at least one Pro.

27. The compound of claim 25 wherein the pair Xaa-Yaa is Pro-D-Pro or D-Pro-Pro.

25

28. The compound of claim 24 wherein X and Y further form a disulfide bridge.

29. The compound of claim 28 wherein:

30 X is  $Xaa_n$ -Ile-Leu-Cys-Asn-;

Y is  $Yaa_n$ -Gly-Lys-Cys-Leu-;

Xaa is any amino acid capable of forming a peptide bond with Yaa;

Yaa is any amino acid capable of forming a

35 peptide bond with Xaa;

and n = 1 to 11.

30. The compound of claim 29 wherein the pair Xaa-Yaa comprises at least one Pro.

31. The compound of claim 29 wherein the pair 5 Xaa-Yaa is Pro-D-Pro or D-Pro-Pro.

32. A recombinant protein in which the CDR-2 region of an immunoglobulin is replaced with the compound of claim 11.

10

33. The protein of claim 32 wherein the immunoglobulin is of a human IgG subclass.

34. The protein of claim 33 wherein the 15 immunoglobulin is a variable light chain ( $V_k$ ).

35. A composition for inhibiting HIV infection which comprises an effective amount of the compound of claim 11 and a pharmaceutically acceptable carrier.

20

36. The method of claim 1 wherein:

A is any amino acid;

B is selected from the group consisting of Gly, Ala, Ser, Thr, Asp, Asn, Glu, Gln, His, Lys and Arg;

25 C is any amino acid;

D is any amino acid;

with the proviso that when X and Y both comprise a naturally occurring amino acid sequence present in the D1 domain of CD4, then X and Y together 30 number less than 31 amino acids.

37. The method of claim 36 wherein X and Y number less than 13 amino acids.

35

38. The method of claim 36 wherein X and Y number less than 5 amino acids.

39. The method of claim 36 wherein X and Y number less than 3 amino acids.

40. The method of claim 36 wherein A is Val.

5

41. The method of claim 36 wherein B is Glu.

42. The method of claim 36 wherein C is Asp.

10

43. The method of claim 36 wherein D is Gln.

44. The method of claim 36 wherein A-B-C-D is Val-Glu-Asp-Gln.

15

45. A conformationally restricted compound of the formula:

X-A-B-C-D-Y

wherein

A is any amino acid;

20

B is selected from the group consisting of Gly, Ala, Ser, Thr, Asp, Asn, Glu, Gln, His, Lys and Arg;

C is any amino acid;

D is any amino acid;

25

the combination X + Y provides a means to restrict the stereochemical structure of A-B-C-D such that

A-B-C-D forms a beta turn;

30

with the proviso that when X and Y both comprise a naturally occurring amino acid sequence present in the D1 domain of CD4, then X and Y together number less than 31 amino acids;

or a pharmaceutically acceptable salt thereof.

46. The compound of claim 45 wherein A is Val.

35

47. The compound of claim 45 wherein B is Glu.

48. The compound of claim 45 wherein C is Asp.

49. The compound of claim 45 wherein D is Gln.

5 50. The compound of claim 45 wherein A-B-C-D is Val-Glu-Asp-Gln.

10 51. The compound of claim 45 wherein X is one or more amino acid; and Y is one or more amino acids.

15 52. The compound of claim 51 wherein X and Y are joined by a disulfide bridge.

15 53. The compound of claim 52 wherein:

15 X is Cys-(X<sub>1</sub>)<sub>n</sub>;  
Y is Cys-(Y<sub>1</sub>)<sub>n</sub>;  
(X<sub>1</sub>) is any amino acid except Pro;  
(Y<sub>1</sub>) is any amino acid except Pro;

20 and n = 0 to 5.

54. The compound of claim 52 wherein:

25 X is selected from the group consisting of Xaa<sub>n</sub>-Cys-, Xaa<sub>n</sub>-Cys-Glu-, Xaa<sub>n</sub>-Cys-Cys-Glu- and Xaa<sub>n</sub>-Cys-Ile-Cys-Glu-;

Y is selected from the group consisting of Yaa<sub>n</sub>-Cys-, Yaa<sub>n</sub>-Cys-Lys-, Yaa<sub>n</sub>-Cys-Glu-Lys- and Yaa<sub>n</sub>-Cys-Glu-Glu-Lys;

30 Xaa is any amino acid except Pro;  
Yaa is any amino acid except Pro;  
and n = 0 to 11

55. The compound of claim 51 wherein X and Y are joined by a peptide bond.

35

56. The compound of claim 55 wherein:

X is selected from the group consisting of

$Xaa_n$ -,  $Xaa_n$ -Cys-Glu- and  $Xaa_n$ -Tyr-Ile-Cys-Glu-;  
Y is selected from the group consisting of

$Yaa_n$ -,  $Yaa_n$ -Glu-Lys- and  $Yaa_n$ -Val-Glu-Glu-Lys-;

Xaa is any amino acid capable of forming a  
5 peptide bond with Yaa;

Yaa is any amino acid capable of forming a  
peptide bond with Yaa;

and n = 1 to 11.

10 57. The compound of claim 56 wherein the pair  
Xaa-Yaa comprises at least one Pro.

58. The compound of claim 56 wherein the pair  
Xaa-Yaa is Pro-D-Pro or D-Pro-Pro.

15 59. The compound of claim 55 wherein X and Y  
further form a disulfide bridge.

20 60. The compound of claim 59 wherein:

X is  $Xaa_n$ -Tyr-Ile-Cys-Glu-;

Y is  $Yaa_n$ -Val-Glu-Cys-Lys-;

Xaa is any amino acid capable of forming a  
peptide bond with Yaa;

Yaa is any amino acid capable of forming a

25 peptide bond with Xaa;

and n = 1 to 11.

61. The compound of claim 60 wherein the pair  
Xaa-Yaa comprises at least one Pro.

30 62. The compound of claim 60 wherein the pair  
Xaa-Yaa is Pro-D-Pro or D-Pro-Pro.

35 63. A recombinant protein in which the CDR-2  
region of an immunoglobulin is replaced with the  
compound of claim 45.

64. The protein of claim 63 wherein the immunoglobulin is of a human IgG subclass.

65. The protein of claim 64 wherein the 5 immunoglobulin is a variable light chain ( $v_k$ ).

66. A composition for inhibiting HIV infection which comprises an effective amount of the compound of claim 45 and a pharmaceutically acceptable 10 carrier.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/08873

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>3</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC (5): Please See Attached Sheet. US CL : Please See Attached Sheet.		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>4</sup>		
Classification System	Classification Symbols	
U.S.	530/317, 321, 323, 324, 325, 326, 327, 328, 329, 330, 350, 387, 389; 514/9, 10, 11, 12, 13, 14, 15, 16, 17, 18	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>5</sup>		
CHEMICAL ABSTRACTS AND BIOLOGICAL ABSTRACTS ONLINE COMPUTER SEARCH		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>14</sup></b>		
Category <sup>6</sup>	Citation of Document, <sup>15</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>
Y	US, A, 4,737,487 (WATTS ET AL.) 12 April 1988. See column 3, lines 45-51 in particular.	12,14,46
P,X/	US, A, 5,001,049 (KLEIN ET AL.) 19 March 1991. See column 1, lines 56-59 in particular.	11, 13, 15-16, 20-23, 35, 45, 51-54/12, 14, 46
Y	Lehninger, "Principles of Biochemistry", published 1982 by Worth Publisher, Inc. (NY). pages 95-117. See Table 5-3 in particular.	12,14,46
Y	Cell, volume 57, issued 05 May 1989, ARTHOS ET AL., "Identification of the residues in human CD4 critical for the binding of HIV", pages 469-481. See pages 469, 470 and 472 in particular.	1-66
X/Y	Science, volume 241, issued 05 August 1988, LIFSON ET AL., "Synthetic CD4 peptide derivatives that inhibit HIV infection and cytopathicity", pages 712-716, see the Abstract in particular.	45-51/1,36-44
<p>* Special categories of cited documents:<sup>16</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search <sup>2</sup>	Date of Mailing of this International Search Report <sup>2</sup>	
03 MARCH 1992	11 MAR 1992	
International Searching Authority <sup>1</sup>	Signature of Authorized Officer <sup>20</sup>	
ISA/US	CHRISTINA CHAN	

**FURTHER INFORMATION CONTINUED FROM PREVIOUS SHEETS**  
(Not for publication)

**I. CLASSIFICATION OF SUBJECT MATTER:**  
IPC (5):

C07K 5/10, 7/06, 7/08, 7/10, 7/54, 7/64, 15/06; A61K 37/02, 37/04, 39/395

**I. CLASSIFICATION OF SUBJECT MATTER:**  
US CL :

530/317, 321, 323, 324, 325, 326, 327, 328, 329, 330, 350, 387, 389; 514/9, 10, 11, 12, 13, 14, 15, 16, 17, 18

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